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Antioxidant potential of aquatic plant *Scirpus mucronatus* found in water bodies of Dinajpur district, Bangladesh

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Abstract

Generation of free radicals cause many problems in human body, but natural defense mechanism minimizes this problem. Synthetic antioxidants are commonly used but it associates with safety concern so antioxidants from natural origin are attracting researcher attention. Bangladesh enriches with many aquatic plants, and few are reported for human consumption and traditional medicine. To examine antioxidant potentiality, 14 (fourteen) aquatic plants were screening against radical scavenging assay and showed significant antioxidant activities. Among them *Scirpus mucronatus* was selected for others antioxidant assay (total phenolic content, radical scavenging, and reducing activity) as there are limited research on this aquatic plant. This aquatic plant showed DPPH radical scavenging activity of $55.61 \pm 0.51\%$, and total phenolic content of 30.53 ± 0.71 mg GAE/g dry weight. ABTS radical scavenging, CUPRAC and FRAP assay exhibited significant antioxidant activity in concentration dependent manner as well. These assay results revealed antioxidant potentiality of *S. mucronatus*. Further experimental works are required to isolate its phenolic compounds and plausible application in different field as antioxidants.

Keywords: antioxidant, aquatic plant, DPPH, ABTS

Introduction

Free radicals are generated in human body by oxidative metabolism. Natural defense mechanism neutralizes free radicals but in excessive condition it attack healthy cell and cause many diseases ^[1]. Antioxidants are used to quench free radicals. Antioxidants are phenolic compound originating from various plants, fruits and vegetables ^[2]. This phenolic compound possesses many medicinal properties such as anti-inflammatory, antimicrobial, antifungal, and anti-hypertensive. However, synthetic antioxidants such as BHA, BHT are also commonly used but their uses are criticized for toxicity problem. Now researchers are searching for alternative antioxidant from natural sources. Bangladesh is a country of ponds, lakes, rivers, canals, wetlands, rice fields and flood plain areas. These waterbodies are enriched with fisheries resources including aquatic plants. Usually, aquatic plants are categorized into four classes: algae, floating plants, submerged plants, and emerged plants ^[3]. In Bangladesh more than 300 species are identified as aquatic plants ^[4]. Most abundant aquatic plants are *Nymphaea nauchali*, *Eichhorina crassipes*, *Pistia stratiotes*, *Lemna minor*, *Azolla pinnata*, and *Ipomea aquatica*. These aquatic plants are essential component of aquatic system and serve as food for animals, birds, and herbivore fish such as grass carp, *Ctenopharyngodon idella*. Few aquatic plants, *Ipomea aquatica* (Kalmi sak) and *Nymphaea* (Sapla) are served as popular dishes in Bangladesh. But generally aquatic plants are completely removed from water bodies in the name of undesirable species during production of fish and rice. However, pharmacological properties of aquatic plants have been reported by some researchers ^[5, 6]. Traditionally *I. aquatica* possess the medicinal properties of anti-inflammatory, anti-diabetic, anti-carminative, treating fever and jaundice diseases. Historically *Nelumbo nucifera* and *Trapa natans* are intensively used in traditional medicine of Asia and Europe. So, literatures suggest that aquatic plants possess medicinal value. In Bangladesh limited research has been conducted on medicinal properties of aquatic plants. In this research 14 aquatic plants of Dinajpur district were screen against DPPH radical scavenging assay and select *S. mucronatus* for further antioxidant test as there are no reports on antioxidant activity of this aquatic plant.

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Materials and Methods

Survey and collection of aquatic plants

Aquatic plants were collected from different waterbodies of Dinajpur district (Chirirbandar and Kaharol upazilla) of Bangladesh. Collected aquatic plants were brought to the laboratory of Fisheries Technology, HSTU, Dinajpur. Aquatic plants washed in running tap water. Identification and voucher specimens were deposited in the lab.

Extract preparation

Aquatic plants were finely cut into small pieces, weighing, and methanol (3 times) were added in glass jar and left in dark place. Extract was decanted after 3 days, and another organic extract was prepared in same way. Both extracts were combined, evaporated, and concentrated in rotary evaporator.

Screening of aquatic plants extract for antioxidant assay

Each aquatic plants extract was screened for antioxidant activity. Radical scavenging assay is the most widely method to determine antioxidant activity. DPPH radical scavenging assay was used to screen all extract of aquatic plants.

DPPH radical scavenging assay

Tested sample solution (50 μ l) in MeOH was added to 40 μ g/ml DPPH radical solution (950 μ l) in MeOH in a test tube and mixed vigorously. In the dark, the mixture was left for 30 min and measured absorbance at 517 nm [7]. The radical scavenging activity was calculated using the following equation:

$$\text{Scavenging activity (\%)} = \{A_0 - A_S / A_0\} \times 100$$

Where A_0 is the absorbance of control, A_S is the absorbance of sample.

Total phenol content (TPC)

TPC of *S. mucronatus* extract was determined according to the method described by Quy Diem Do *et al.* [8] with minor modifications. Methanol was used to dissolve the extract to a concentration of 50 μ g/mL. The gallic acid at concentration of 0-60 μ g/mL was prepared to set the calibration curve. The diluted extract or gallic acid (1.6 mL) was added to 0.2 mL Folin-Ciocalteu reagent (5-fold diluted with distilled water) and mixed thoroughly for 3 minutes. Sodium carbonate (0.2 mL, 10% w/v) was added to the mixture and the mixture was allowed to stand for 30 minutes at room temperature. The absorbance of the mixture was measured at 760 nm using a UV-VIS spectrophotometer. TPC was expressed as milligram gallic acid equivalent per gram of *S. mucronatus* (mg GAE/g).

ABTS Radical Scavenging Assay

ABTS radical cation solution was prepared by adding 2.45 mM (final concentration) potassium persulfate to 7 mM ABTS in water and kept overnight in dark place [9]. Before assay, the cation solution was diluted with ethanol to absorbance of 0.70 at 734 nm. Tested sample solution (10 μ l) was added to the ABTS radical solution (1.0 ml). The mixture was left at room temperature for 10 min and absorbance was recorded at 734 nm. The antioxidant activity of each sample was calculated from following equation:

$$\text{Antioxidant activity (\%)} = \{A_0 - A_S / A_0\} \times 100$$

Where A_0 is control absorbance, A_S is sample absorbance.

Cupric Reducing Antioxidant Capacity (CUPRAC) Assay

Tested sample solution (0.1 ml) was added to the premixed reaction mixture containing 10 mM CuCl_2 solution (0.25 ml), 7.5 mM ethanolic neocuproine solution (0.25 ml), and 1 M ammonium acetate buffer solution (0.25 ml, pH 7.0) in each tube. The mixture was incubated for 30 min at room temperature, then absorbance was measured at 450 nm [10].

Ferric Reducing Antioxidant Power (FRAP) Assay

Each tube contained freshly prepared FRAP reagent by mixing 300 mM sodium acetate buffer (750 μ l, pH 3.6), 10 mM TPTZ in 40 mM HCl (75 μ l) and of 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (75 μ l). Then sample solution (30 μ l) was added along with water (100 μ l) to the premixed FRAP reagent. The mixture was left for 4 min at room temperature and absorbance was measured at 593 nm [11].

Statistical Analysis

All the assay was performed in triplicate and mean value \pm standard deviation expressed for each case.

Results and Discussion

Screening of aquatic plants

DPPH radical scavenging assay is commonly used in testing antioxidant activity by radical scavenging capacity of the plants extract [12]. DPPH generates violet color in methanol due to presence of unpaired nitrogen electron and reduced to yellow by antioxidant compound [13]. So, low absorbance indicates scavenging ability of the tested sample. Collected aquatic plants were screened for antioxidant activity by this method. All aquatic plants showed significant DPPH radical scavenging activity (Table 1). Among the 14 aquatic plants, *P. hydropiper* and *S. mucronatus* exhibited the highest and lowest activity of 89.54 \pm 0.69% and 55.61 \pm 0.51% at concentration of 250 μ g/ml, respectively. Two human consumed aquatic plants *E. fluctuans* and *I. aquatica* showed DPPH scavenging activity of 84.80 \pm 0.70% and 79.52 \pm 0.87%, respectively exhibited relatively similar scavenging trend as described by Simlai, A. *et al.* [12]. All collected aquatic plants showed significant DPPH radical scavenging activity because of their polyphenolic content and other secondary metabolites. Despite low scavenging activity, *S. mucronatus* was selected for further antioxidant assay as there is limited antioxidant research on this aquatic plant.

Table 1: DPPH screening results for antioxidant activity.

No.	Local name	Scientific name	DPPH radical scavenging activity (%) at 250 μ g/ml
1.	Bishkatali	<i>Polygonum hydropiper</i>	89.54 \pm 0.69
2.	Kochu	<i>Colocasia esculenta</i>	78.36 \pm 0.58
3.	Chechra	<i>Scirpus mucronatus</i>	55.61 \pm 0.51
4.	Kanai nala	<i>Commelina axillaris</i>	85.89 \pm 0.75
5.	Helencha	<i>Enhydra fluctuans</i>	84.80 \pm 0.70
6.	Topa pana	<i>Pistia stratiotes</i>	86.35 \pm 0.34
7.	Khudi pana	<i>Lemna minor</i>	85.29 \pm 0.42
8.	Lal Shapla	<i>Nymphaea rubra</i>	86.05 \pm 0.54
9.	Shada Shapla	<i>Nymphaea nauchali</i>	82.63 \pm 1.14
10.	Kochuripana	<i>Eichhornia crassipes</i>	82.44 \pm 0.69
11.	Kalmilata	<i>Ipomoea aquatica</i>	79.52 \pm 0.87
12.	Kesordam	<i>Jussiaea repens</i>	87.66 \pm 0.87
13.	Malancha	<i>Alternanthera philoxeroides</i>	77.96 \pm 0.47
14.	Sushni Shak	<i>Marsilea quadrifolia</i>	59.04 \pm 5.31
	Butylatedhydroxy toluene (BHT)		98.41 \pm 0.81

Each value is the mean \pm standard deviation from three replicates.

Total phenol contents (TPC)

TPC indicates the phenolic contents present in *S. mucronatus*, and Folin Ciocalteu method was used to calculate quantitative determination of phenolic content in term of gallic acid equivalent. TPC values were calculated from the calibration curve (Figure 1) and found 30.53 ± 0.71 mg GAE/g dry weight which was higher than *L. minor* at 16.7 ± 0.0 and *I. Aquatica*

at 13.75 ± 0.64 mg GAE/g dry weight, respectively but much lower than *E. fluctuans* at 61.85 ± 1.53 mg GAE/g dry weight [12, 14]. In any plant extracts, phenolic compounds mainly responsible for antioxidant activity, provide protection in adverse condition, pest and disease resistance as well [15]. Due to health beneficial role of this phenolic compounds, its carry potentiality for pharmaceutical industry [16].

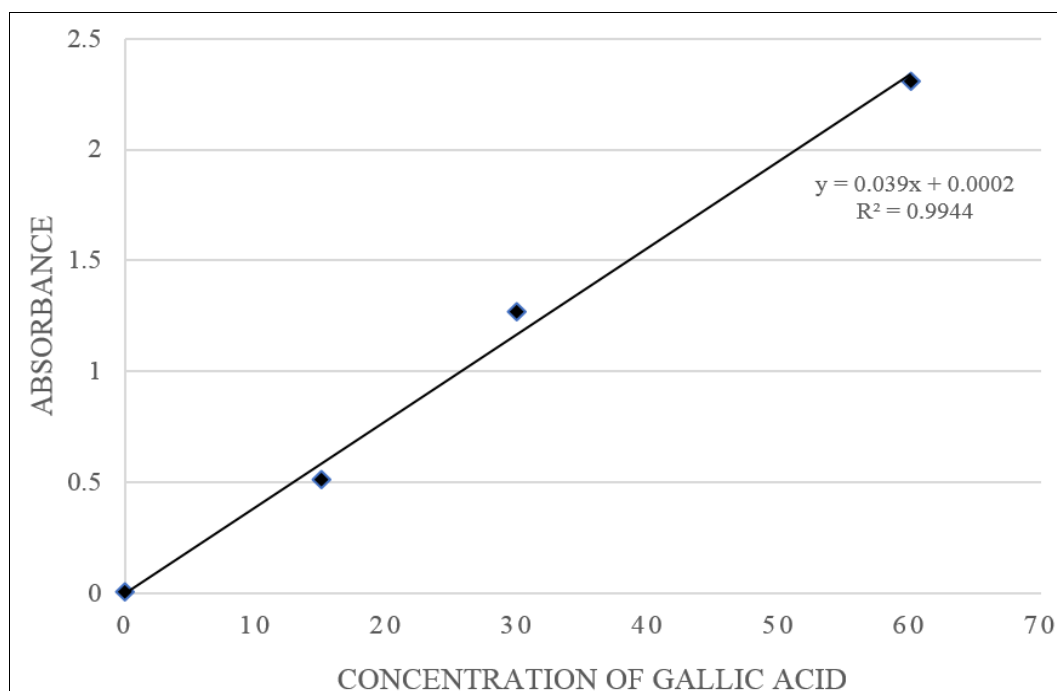


Fig 1: Standard calibration curve to determine phenolic content.

ABTS radical scavenging assay relies on decolorization of radical cation prior to reaction with putative antioxidants. Blue/green colored ABTS radical cation generated through the reaction between ABTS solution and potassium persulfate. Methanolic extract of *S. mucronatus* showed effective

ABTS⁺ radical scavenging activity (Figure 2) in a concentration-dependent basis (0-50 μ g/ml). There is a significant decrease of ABTS⁺ due to increasing concentration of *S. mucronatus* extract. Similar ABTS⁺ radical scavenging trends found in *L. minor* [14].

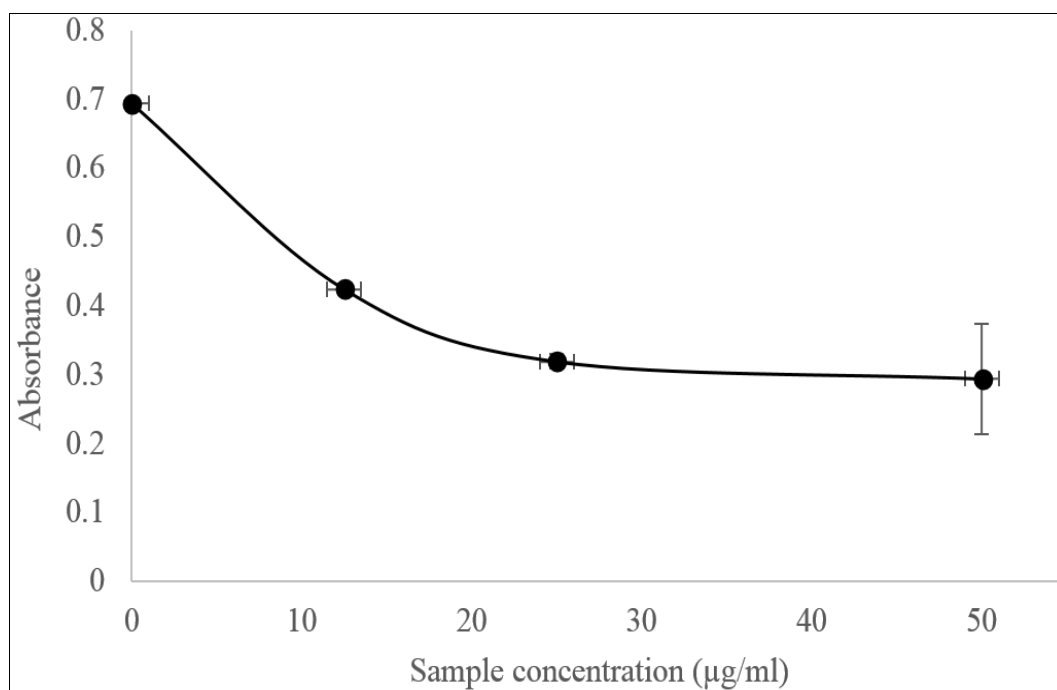


Fig 2: ABTS⁺ radical scavenging activity at different concentrations (0-50 μ g/ml) of *S. mucronatus* extract.

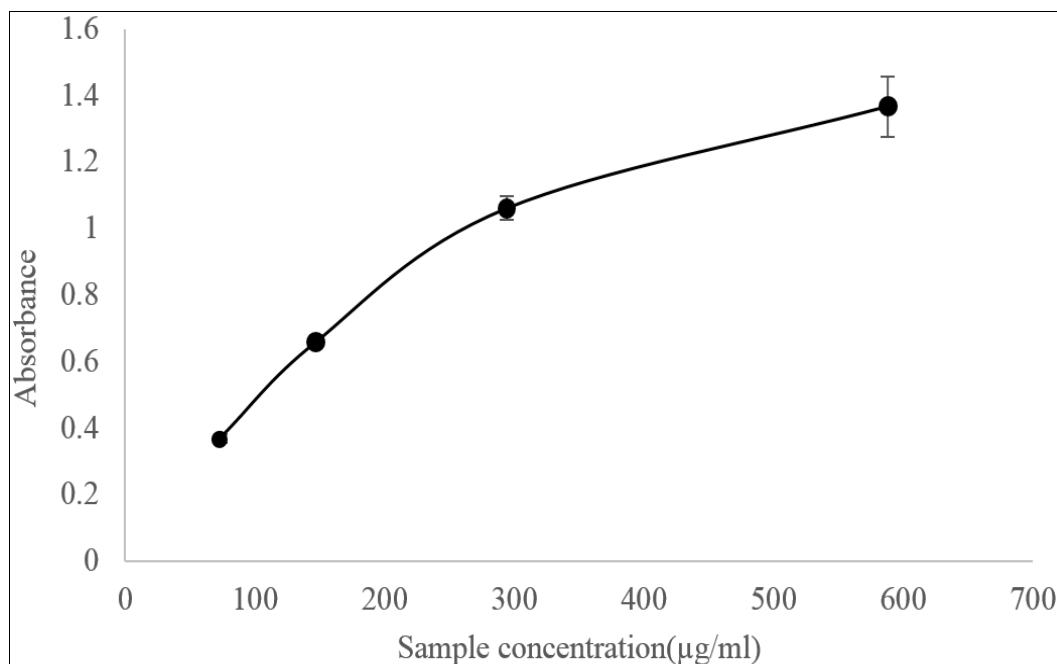


Fig 3: CUPRAC activity of *S. mucronatus*

The antioxidant potentiality can also be tested by examining reducing power along with other mechanism such as chain initiation prevention, chelating metal ion catalysts, degradation of peroxide, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging^[17, 18].

Figure 3 and 4 showed reducing power of *S. mucronatus* in concentration dependent manner. The reducing power is associated with presence of reductants breaking free radical chain by donating a hydrogen atom^[19]. Higher absorbance indicates greater reducing power.

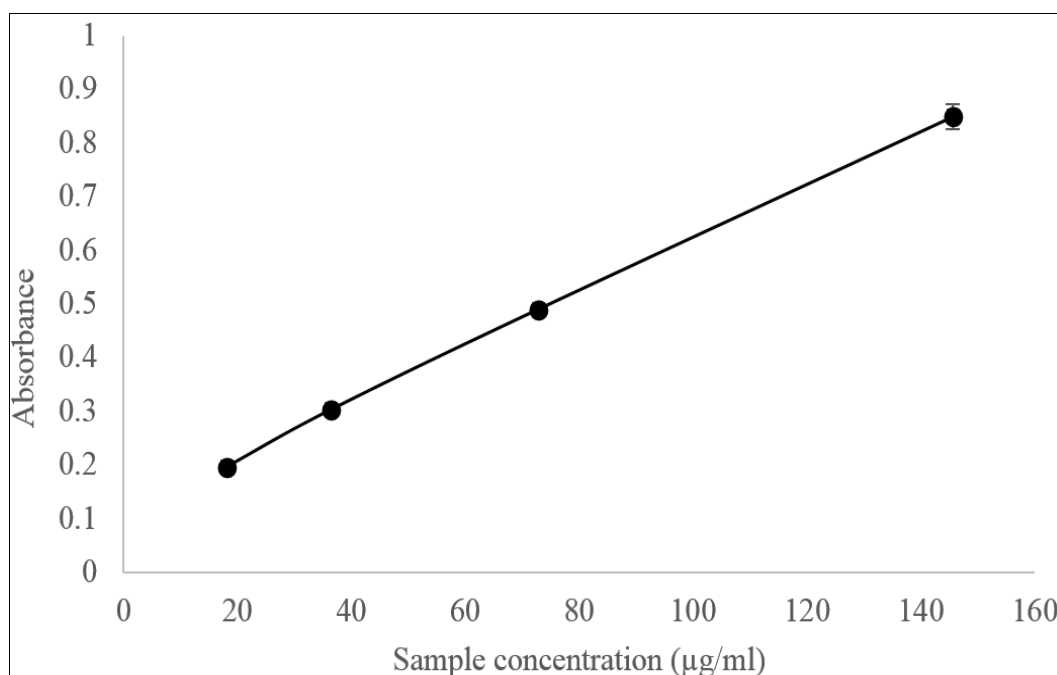


Fig 4: FRAP activity of *S. mucronatus*

Conclusion

S. mucronatus is a commonly found aquatic plant in waterbodies of Dinajpur district. Usually, this aquatic plant is treated as undesirable species with no economic value. But this research investigated its medicinal value such as antioxidative potentiality. Various antioxidant assay showed encouraging results indicating its phenolic compounds can replace synthetic toxic antioxidant used in pharmaceutical, food, and cosmetic industry. Further experimental works are required to isolate its phenolic compounds and plausible application in different field as antioxidants.

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